



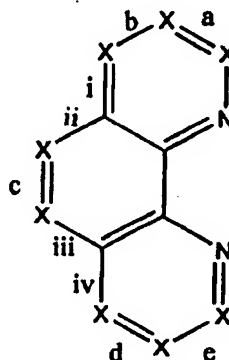
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification <sup>6</sup> :  <b>C07D 471/04, 471/14, C09K 11/00, C07D 487/14 // (C07D 471/04, 221:00, 221:00) (C07D 471/14, 241:00, 221:00, 221:00) (C07D 487/14, 241:00, 241:00, 241:00)</b></p>	<p><b>A1</b></p>	<p>(11) International Publication Number: <b>WO 98/49163</b></p> <p>(43) International Publication Date: 5 November 1998 (05.11.98)</p>
<p>(21) International Application Number: PCT/EP98/02359</p> <p>(22) International Filing Date: 20 April 1998 (20.04.98)</p> <p>(30) Priority Data:            97201236.3 25 April 1997 (25.04.97) EP            (34) Countries for which the regional or international application was filed: NL et al.</p> <p>(71) Applicant (for all designated States except US): AKZO NOBEL N.V. [NL/NL]; Velperweg 76, NL-6824 BM Arnhem (NL).</p> <p>(72) Inventors; and            (75) Inventors/Applicants (for US only): VERHOEVEN, Jan, Willem [NL/NL]; Westerzoom 34, NL-1541 TW Koog aan de Zaan (NL). VAN DER TOL, Erik, Boudewijn [NL/NL]; Laan der Nederlanden 377, NL-1945 RK Beverwijk (NL). STEEMERS, Frank, Johannes [NL/NL]; Zeelandstraat 111, NL-7543 VC Enschede (NL). VERBOOM, Willem [NL/NL]; De Nachtegaal 6, NL-7671 WB Vriezenveen (NL). REINHOUDT, David, Nicolaas [NL/NL]; De Voskamp 7, NL-7552 GD Hengelo (NL). HOFSTRAAT, Johannes, Willem [NL/NL]; Marjoleinveld 4, NL-7006 VG Doetinchem (NL).</p>		<p>(74) Agent: SCHALKWIJK, Pieter, Cornelis; Akzo Nobel N.V., Patent Dept. (Dept. APTA), P.O. Box 9300, NL-6800 SB Arnhem (NL).</p> <p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b>  <i>With international search report.            Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>

(54) Title: COMPLEX COMPRISING A RARE-EARTH METAL ION AND A COMPLEXING MOIETY

(57) Abstract

The invention pertains to a complex comprising a rare-earth metal ion and the complexing moiety of general formula (I) characterized in that X is independently CH or N, preferably at least one of the groups X being N, and the bonds a, b, c, d, and e, and the combination of bonds i/ii and iii/iv are optionally condensed with a benzene group or a condensed aromatic moiety, wherein aromatic carbon atoms may be replaced by nitrogen atoms and wherein the complexing moiety may be substituted with C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>3-4</sub> alkylene, CN, halogen, COOH, C<sub>1-3</sub> alkyl-COOH, NO<sub>2</sub>, NH<sub>2</sub>, or a pending group for further functionalization or complexation, with the proviso that the complexing moiety is not 1,10-phenanthroline.



(I)

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Licchtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## COMPLEX COMPRISING A RARE-EARTH METAL ION AND A COMPLEXING MOIETY

- 5 The invention pertains to a complex comprising a rare-earth metal ion and a complexing moiety, a process for the preparation of the same, an optical device, and a diagnostic kit comprising said complex.

10 Molecular systems that combine specific binding ability and long-lived luminescence are of great interest for application as chemosensors and luminescence probes. Lanthanide ions in particular are of interest, because of their relatively long luminescence lifetime (up to ms) and their emission in the visible part of the spectrum. Such lanthanide based probes have been used for a wide range of applications. The disadvantage of the low extinction

15 coefficient of lanthanide ions can be overcome by application of a sensitizer ("antenna") moiety. Recently, also near-IR luminescent lanthanide probes have been proposed, which make use of  $\text{Nd}^{3+}$  or  $\text{Yb}^{3+}$  as luminophores, both ions which emit in the region beyond 800 nm and are excitable in the visible part of the spectrum. With the same approach the  $\text{Er}^{3+}$  ion, which gives

20 luminescence in the 1530 nm range, could be excited. Such complexes are also of interest for application in optical devices, such as optical amplifiers and lasers.

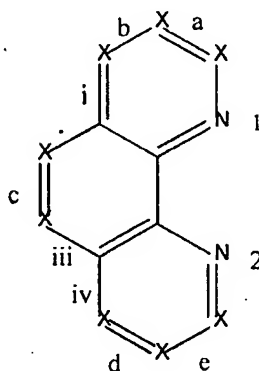
The challenge is to optimize this process in such a way that the sensitizer is

25 able to sensitize multiple lanthanide ions ( $\text{Eu}^{3+}$ ,  $\text{Tb}^{3+}$ ,  $\text{Sm}^{3+}$ ,  $\text{Dy}^{3+}$ ,  $\text{Nd}^{3+}$ ,  $\text{Yb}^{3+}$ ,  $\text{Er}^{3+}$ , etc.) with high luminescence efficiencies, allowing multi-probe analysis. Moreover, the sensitizer should have an absorption in the near-UV/VIS area to allow the use of inexpensive glass optics and simple excitation sources (e.g., Hg-lamp). In view of the relatively short wavelength of  $\text{Tb}^{3+}$  this allows

30 only a small excitation window for effective sensitization. The sensitization of

Tb<sup>3+</sup> in particular is desirable, because of its high intrinsic quantum yield and its relatively low susceptibility to non-radiative quenching effects.

- In European patent application EP 493,745 a fluorescent compound has been disclosed which forms a complex with rare-earth metal ions. Such compounds are macro-cyclic compounds containing two phenanthrene moieties forming a cage structure in which the rare-earth metal ion can be complexed. These compounds were obtained by relatively complex synthetic methods, and it further appeared that they have a limited applicability and sometimes low quantum yields. There is, however, a need for simple compounds which strongly complex with rare-earth metal ions, have high quantum yields, and can be used for IR as well as for UV luminescence.
- It has now been found that suitable complexes are complexes comprising a rare-earth metal ion and the complexing moiety of the general formula:



characterized in that

- X is independently CH or N, and the bonds a, b, c, d, and e, and the combination of bonds i/ii and iii/iv are optionally condensed with a benzene group or a condensed aromatic moiety, wherein aromatic carbon atoms may be replaced by nitrogen atoms and wherein the complexing moiety may be substituted with C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>3-4</sub> alkylene,

CN, halogen, COOH, C<sub>1-3</sub> alkyl-COOH, NO<sub>2</sub>, NH<sub>2</sub>, or a pending group for further functionalization or complexation, with the proviso that the complexing moiety is not 1,10-phenanthroline. Preferably at least one the groups X is N.

5

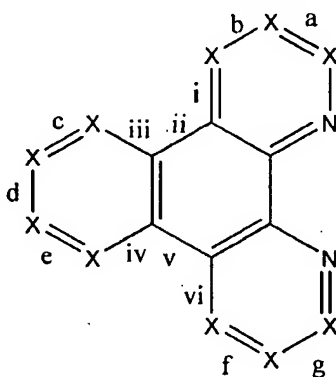
The N atoms numbered 1 and 2 act as coordinating groups for lanthanide ions, ensuring the shortest possible distance between the sensitizer and the acceptor ions, which favors energy transfer to the lanthanide ion in comparison to non-radiative quenching mechanisms. Moreover, the triplet  
10 state of a sensitizer will not change dramatically upon the incorporation of N-ligating atoms, leaving the reported beneficial characteristics of the complexing moiety intact. The ligating properties of the diaza-moiety of the complexing moiety can be applied both for direct complexation of the rare earth ions (in which case a 2:1 complex is formed, with two diaza-ligands  
15 bound to the ion) or for indirect complexation, in which case the sensitizing compound is attached to another ligating moiety, such as a calixarene, an EDTA derivative, a pyridine dicarboxylic acid derivative, a dithiocarbamate derivative or a  $\beta$ -diketonate derivative.

20 Extensive studies aimed at direct coordination have made use of bipyridine-type complexes. Lehn et al., Angew. Chem., Int. Ed. Engl., 29 (1990), 1304, employed cryptates in which bipyridine ("the antenna") is incorporated into the macrocyclic structure. However, in aqueous solution the Eu<sup>3+</sup> and Tb<sup>3+</sup> cryptates convert only about 1% of the incident UV  
25 photons into emitted visible photons. Hemmilä et al., Helv. Chim. Acta., 76 (1993), 1361 investigated terpyridine structures and picolinic acid derivatives (J. Chem. Soc., Perkin Trans., 2 (1995), 995) in which the coordinating pyridine moiety serves as sensitizer, giving high quantum yields for Eu<sup>3+</sup> and Tb<sup>3+</sup>.

30

It has now been found that in addition to the N atoms numbered 1 and 2, the addition of at least one other N atom in one of the aromatic nuclei, and preferably two other N atoms in the aromatic nuclei, considerably improves the quantum yield of the complex and the extinction coefficient of the sensitizer.

The presently claimed compounds preferably comprise a rare-earth metal ion and the complexing moiety of the general formula:

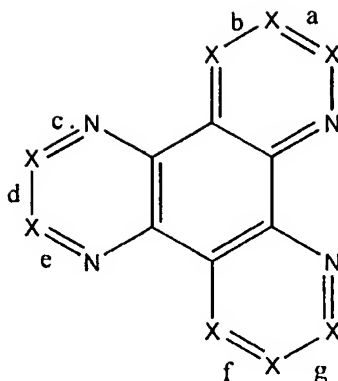


- 10 wherein X is independently CH or N, preferably at least one of the groups X being N, and the bonds a, b, c, d, e, f, and g, and the combination of bonds i/ii/iii and iv/v/vi are optionally condensed with a benzene group or a condensed aromatic moiety, wherein aromatic carbon atoms may be replaced by nitrogen atoms and wherein the complexing moiety may be
- 15 substituted with C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>3-4</sub> alkylene, CN, halogen, COOH, C<sub>1-3</sub> alkyl-COOH, NO<sub>2</sub>, NH<sub>2</sub>, or a pending group for further functionalization or complexation. More preferred are the complexes wherein two of the groups X are N.

Most preferred is the complex comprising a rare-earth metal ion and the

20 complexing moiety of the general formula:

5



wherein X is independently CH or N, and the bonds a, b, c, d, e, f, and g are optionally condensed with a benzene group or a condensed aromatic moiety, wherein aromatic carbon atoms may be replaced by nitrogen atoms and wherein the complexing moiety may be substituted with C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>3-4</sub> alkylene, CN, halogen, COOH, C<sub>1-3</sub> alkyl-COOH, NO<sub>2</sub>, NH<sub>2</sub>, or a pending group for further functionalization or complexation.

The term C<sub>1-6</sub> alkyl means a branched or unbranched alkyl group with 1 to 6 carbon atoms, such as methyl, ethyl, propyl isopropyl, butyl, neopentyl, and hexyl. The term C<sub>1-3</sub> alkyl in C<sub>1-3</sub> alkyl-COOH means methyl, ethyl, or propyl. Methyl is the preferred alkyl group.

The terms C<sub>2-6</sub> alkenyl and C<sub>2-6</sub> alkynyl mean, respectively, an alkenyl and alkynyl group with 2 to 6 carbon atoms. Examples are ethenyl, propenyl, ethynyl, propynyl, and the like.

The C<sub>3-4</sub> alkylene group together with the aromatic group to which it is bonded forms a 5- or 6-membered ring.

The term halogen means fluorine, chlorine, bromine, or iodine.

The term pending group for further functionalization or complexation means any pending group which is used in the art to couple with peptides, proteins, carbohydrates, nucleotides, and the like, or which are able to form a complex with rare-earth metal ions. Such pending groups possess an atom or atoms (coupling moiety) which can be used to couple with other

groups or a group or groups which can be used to complex with rare-earth metal ions. Usual coupling moieties are, for instance, isothiocyanate, activated ester (e.g., N-hydroxysuccinimidylester), epoxide, acetic anhydride, and maleimide for coupling to amino groups, amino for coupling  
5 to isothiocyanate, activated ester, epoxide, acetic anhydride, and maleimide groups, maleimide and halogenoacetamide for coupling to thiol groups, thiol for coupling to maleimide and halogenoacetamide groups, hydrazide for coupling to aldehyde groups, aldehyde for coupling to hydrazide groups, isocyanate and acid chloride for coupling to hydroxy,  
10 amino, thiohydroxy, and carboxylic groups, and hydroxy for coupling to isocyanate and acid chloride groups. Groups that can form complexes with rare-earth metal ions are carboxylic acids, and nitrogen, sulfur, and phosphorous containing groups. It is of particular advantage to use pending groups which comprise calixarene, or similar groups.

15

The condensed aromatic group is a group such as naphthalene, phenalene, naphthacene, perylene, pyrene, phenanthrene, anthracene, acenaphthylene, indene, benzanthracene, naphthoperylene, and the like. Preferably, the complexing moiety is condensed with a benzene or a naphthalene group.

20

The complexes can be applied in optical devices and diagnostic kits. Alternative applications for the described complexes are in optical amplification or as active material in a laser, or for security printing (bank notes etc.).

25

The invention is further illustrated by the following examples.



### Example 1

#### Synthesis of Diazatriphenylene

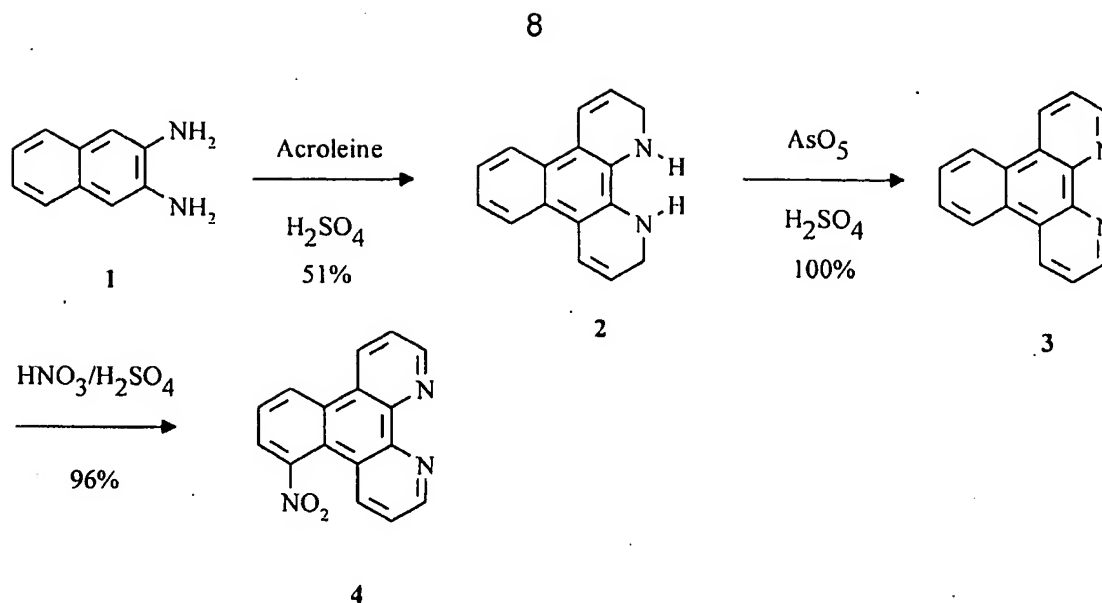
Incorporation of N atoms in a cis-facile manner into the triphenylene structure gives a way to coordinate lanthanide atoms. To this end diazatriphenylene (3) was synthesized using a slightly modified literature procedure (Thang, D. C.; Jacquignon, P.; Dufour, M. J. Heterocyclic Chem., 13 (1976), 641) via a double Skraup reaction on 2,3-diaminonaphthalene (1). Twice performing a Michael addition on acroleine (obtained by dehydration of glycerol with sulfuric acid), followed by an acid catalyzed cyclization, gave diamine (2) in 51% yield. Diamine (2) was oxidized with arsenic pentoxide to give diazatriphenylene (3) in quantitative yield (Scheme 1). Functionalization of 3 via a nitration reaction to obtain nitrodiazatriphenylene (4) was performed using literature procedures (Richardson, F. S. Chem. Rev., 82 (1982), 541).

15

A detailed description of the synthesis follows:

To concentrated sulfuric acid (15 ml) was added dropwise dry glycerol (5 g). The mixture was stirred at room temperature for 5 min. Diamino-2,3-naphthalene (5 g) was added in portions. During this addition the mixture was kept in the liquid state by heating with a metal bath. After the addition was complete arsenic pentoxide (6.5 g) and sulfuric acid (30 ml) were added. The mixture was heated at 130-140°C for 2 h. The mixture was allowed to cool down to room temperature and poured out in water (100 ml) and crushed ice (100 g). The pH of the solution was adjusted to 9. The brown precipitate was filtered off, whereupon the residue was dissolved in ethyl acetate (100 ml). The organic solution was flushed over an aluminum oxide column (neutral, eluent: ethyl acetate). The product was obtained as a yellow solid: mp. 183-184°C (lit. 183°C).

25



Scheme 1: Synthesis of Diazatriphenylene (3)

5

### Example 2

Synthesis of tetraazatriphenylenes and derivatives from 1,10-phenanthroline-4,5-quinone

- 10 The tetraazatriphenylene derivatives (6-11) were synthesized starting from 1,10-phenanthroline-4,5-quinone (5), obtained from the oxidation of 1,10-phenanthroline monohydrate with bromine (*in situ* generated from sulfuric acid and sodium bromide) and nitric acid, according to literature procedures (Gillard, R. D.; Hill, R. E. E.; Maskill, R. J. Chem. Soc. (A) 1970, 1447).
- 15 Subsequently the Schiff-base was formed with the diamines *o*-diaminobenzene, *cis*-diaminocyclohexane, diaminomalononitrile or ethylenediamine, and 1,10-phenanthroline-4,5-quinone in ethanol (or tetrahydrofuran), which after an oxidation step for compounds 6, 8, 10 and 11 (oxygen from the air), provided the tetraazatriphenylenes (6-11) in yields
- 20 ranging from 80-84%. Subsequent heating of the diol with triethylamine as a base in N,N-dimethylformamide (DMF) gave the dicyano-tetraazatriphenylene (7) in 80% yield.

The following products were prepared:

*Dipyrido[3,2-f:2',3'-h]quinoxaline(6)*

To a solution of 1,10-phenanthroline-4,5-quinone (0.60 g, 2.85 mmoles) in tetrahydrofuran (600 ml) was added dropwise ethylenediamine (0.19 g, 2.85 mmoles) dissolved in tetrahydrofuran (10 ml). Molecular sieves (3Å) were added, and the solution was refluxed for 4 h. The solution was concentrated *in vacuo*. The slightly yellow solid was dissolved in dichloromethane (100 ml) and washed with 1N hydrochloric acid (50 ml). The aqueous solution was adjusted to pH 9.0 and cooled on an ice bath. The precipitate was filtered, and after column chromatography (aluminum oxide, dichloromethane) and trituration with benzene compound 6 was obtained as a pure white solid: Yield 92%; mp > 300°C.

15 *2,3-Dicyano-dipyrido[3,2-f:2',3'-h]quinoxaline(7)*

To a solution of 1,10-phenanthroline-4,5-quinone (0.85 g, 4.0 mmoles) in tetrahydrofuran (100 ml) was added dicyanomaleonitrile (0.43 g, 4.0 mmoles). Molecular sieves (3Å) were added, and the solution was refluxed for 1 h. The solution was filtered and concentrated *in vacuo*. The slightly red solid was triturated with dichloromethane. The solid was re-dissolved in DMF (50 ml), and 2 equivalents triethylamine were added under heating at 90°C for 30 min. The solvent was removed *in vacuo*. The solid was re-dissolved in dichloromethane (100 ml) and washed with 1 N hydrochloric acid (100 ml). Compound 7 was obtained after column chromatography (aluminum oxide, dichloromethane) and trituration with hexane/dichloromethane as a pure white solid: Yield 71%; mp > 300°C.

*Cis-2,3-cyclohexane-dipyrido[3,2-f:2',3'-h]quinoxaline(8)*

To a solution of 1,10-phenanthroline-4,5-quinone (0.99 g, 4.7 mmoles) in ethanol was added *cis*-diaminocyclohexane (0.53 g, 4.6 mmoles). Molecular

sieves (3Å) were added, and the solution was refluxed for 1 h. The solution was concentrated *in vacuo*, and the obtained solid was subjected to column chromatography (aluminum oxide, dichloromethane). The solid was triturated with dichloromethane/hexane and crystallized from benzene to obtain  
5 compound 8 as a white pure solid: Yield 86%; mp 292-295°C.

*2,3-(1,2-diaminobenzene)-dipyrido[3,2-f:2',3'-h]quinoxaline(9)*

Compound 9 was prepared and purified as described for compound 8, starting from 1,10-phenanthroline-4,5-quinone (0.6 g, 2.9 mmol) and 1,2-  
10 diaminobenzene (0.3 g, 2.9 mmol). Yield 81%.

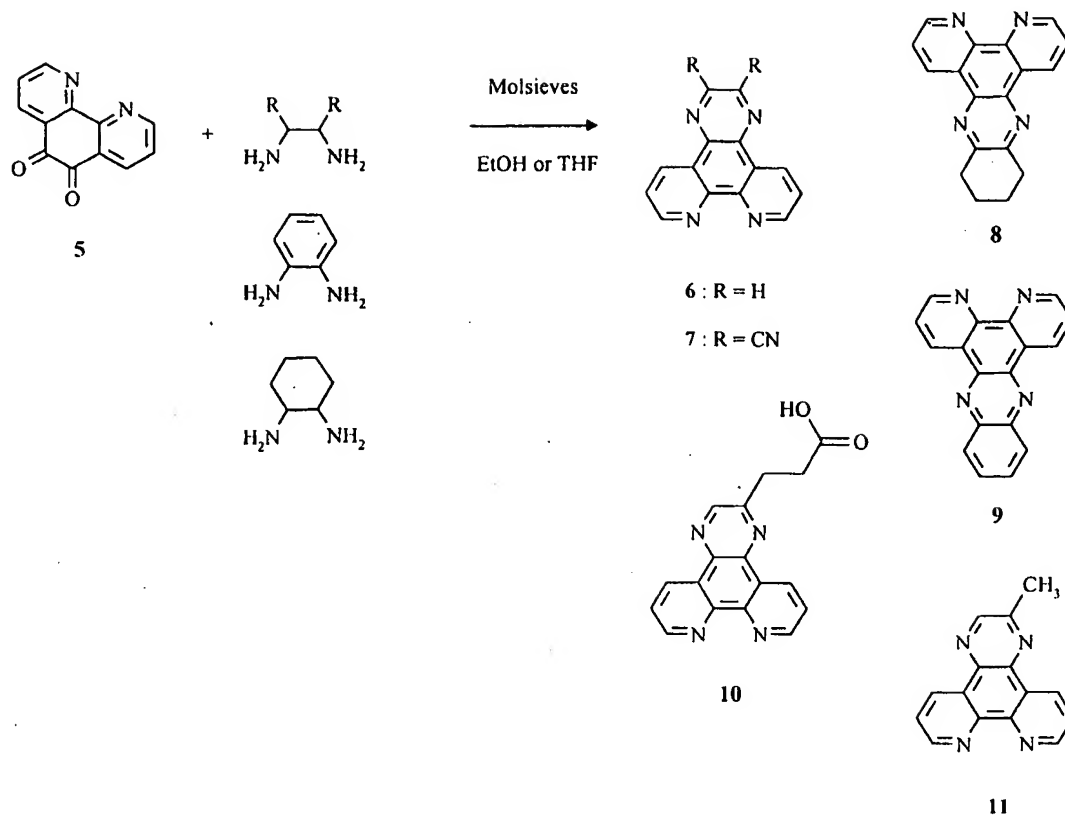
*3-[2-dipyrido[3,2-f:3',2'-h]quinoxaline]proponic acid (10)*

To a solution of 1,10-phenanthroline-4,5-quinone (50 mg, 0.24 mmol) in ethanol (150 ml) was added DL-γ-ornithine (48 mg, 0.24 mmol). The  
15 solution was refluxed for 4 h and concentrated *in vacuo*. The solid was dissolved in ethanol/water (3/1) and purified by column chromatography (aluminum oxide, eluent ethanol/water : 3/1 + 1% triethylamine). The slightly brown solid was triturated with ethyl acetate to obtain 10 as a pure white  
solid: Yield 53 %.

20

*2-Methyl-dipyrido[3,2-f:2',3'-h]quinoxaline(11)*

Prepared in the same way as described for compound 8. Yield 83 %; mp. 271°C.



*Scheme 2 : Synthesis of tetraazatriphenylene derivatives (6-11)*

5

### Example 3

Monofunctionalization of tetraazatriphenylene with 1,10-phenanthroline-4,5-quinone as starting material

- 10 Tetraazatriphenylene (16) was obtained by the reaction of diamine (15) and 1,10-phenanthroline-4,5-quinone 5 in a yield of 83%. For this purpose the diamine (15) was synthesized starting from DL-2,3-diaminopropionic acid (12) via a Boc-protection (13)-amidation (14)-Boc-deprotection (15) sequence as depicted in Scheme 3.

The detailed reaction is as follows:

*D,L-N,N'-di-tert-Boc-2,3-diaminoproponicacid (13).*

To a solution of D,L-2,3-diaminoproponicacid (12) (5.0 g) and triethylamine  
5 (18 g, 0.18 mmole) in 1,4-dioxane and water (75 ml, 1/1) was added di-*tert*-  
butyl dicarbonate (17.08 g, 78.3 mmole). The solution was stirred at room  
temperature for 10 h. After completion of the reaction ethylacetate (100 ml)  
and 1 N hydrochloric acid (100 ml) were added. The organic layer was  
washed with brine (2 × 100 ml). The solvent was dried and removed *in*  
10 *vacuo*, whereupon product 13 was obtained as a white solid: Yield 91%; mp  
162-164°C.

*D,L-N,N'-di-tert-Boc-2,3-diaminoproponicacid dodecyl amide (14)*

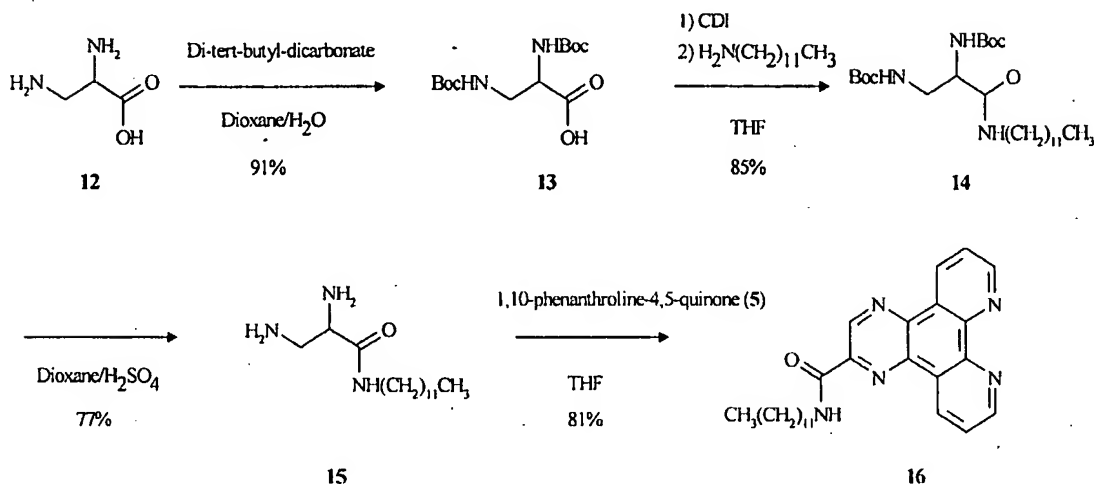
To a solution of acid (13) (4.1 g, 13.3 mmole) in tetrahydrofuran was added  
15 1,1'-carbonyldiimidazole (3.0 g, 13.3 mmole), 4-dimethylaminopyridine  
(catalyst, 0.1 g), and dodecylamine (2.7 g, 13.3 mmole). The solution was  
stirred overnight and concentrated *in vacuo*. To the residue was added  
dichloromethane (100 ml), and the whole was washed with 1 N hydrochloric  
acid (aq.), followed by a standard work-up. The product was purified by  
20 column chromatography (silica gel, dichloromethane) to obtain pure 14 in  
85% yield; mp 103-105°C.

*D,L-2,3-Diaminoproponicacid dodecyl amide (15).*

To a solution of 10% sulfuric acid/dioxane (75 ml) was slowly added 14 (0.80  
25 g, 1.7 mmole) at 0°C. After the addition was complete, the mixture was  
stirred at room temperature for 4 h. The formed precipitate was filtered,  
dissolved in dichloromethane (100 ml), and washed subsequently with 1M  
sodium hydroxide (aq) (50 ml) and brine (50 ml), followed by a standard  
work-up. The product was obtained, after trituration with MeOH, as a white  
30 pure solid: mp. 88-90 °C; Yield 77%.

*2-Dipyrido[3,2-f:2',3'-h]quinoxalineacid dodecyl amide (16).*

Prepared and purified similar to compound 8 starting from 1,10-phenanthroline-4,5-quinone (0.50 g, 1.06 mmoles) and diamine 15 (0.50 g, 1.06 mmoles). Yield 81%; mp. 172-174°C.



*Scheme 3: Synthesis of Monofunctionalized Tetraazatriphenylene 16*

10

Example 4

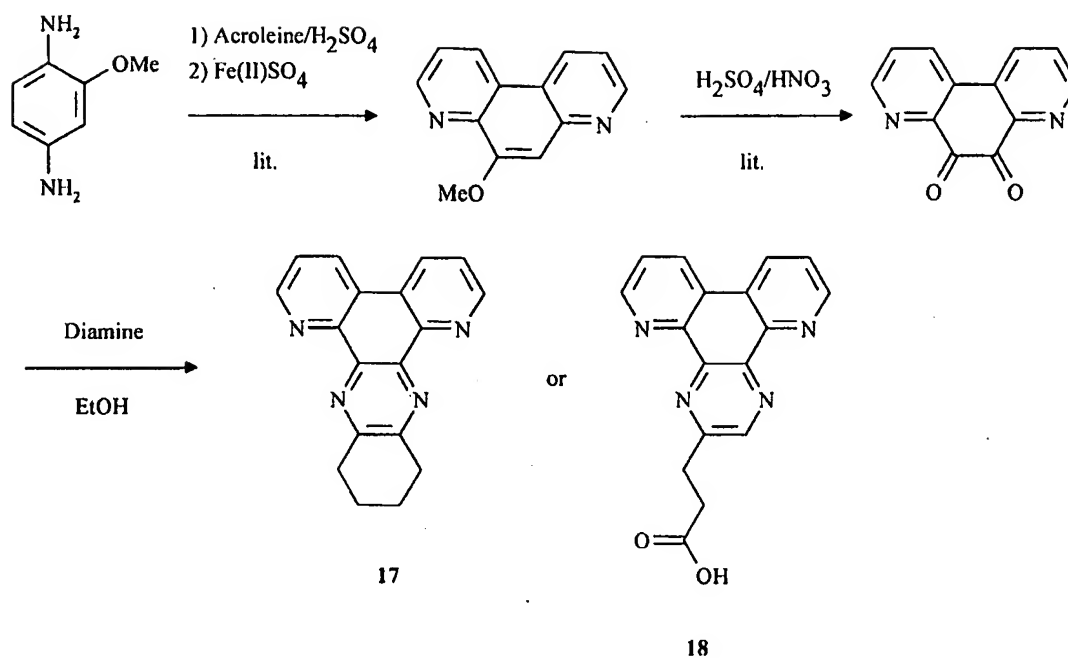
Synthesis of tetraazatriphenylenes from 4,7-phenanthroline-4,5-quinone

*Cis-2,3-cyclohexane-dipyrido[2,3-f:3',2'-h]quinoxaline (17)*

15 Prepared according to a literature procedure starting from 1,2-*cis*-diamino-cyclohexane (0.53 g, 4.6 mmole) and 4,7-phenanthroline-4,5-quinone (0.99 g, 4.7 mmoles).

*3-[2-dipyrido[2,3-f:3',2'-h]quinoxaline]proponic acid (18).*

20 Prepared similar to compound 16 starting from 4,7-phenanthroline-4,5-quinone (50 mg, 0.24 mmole) and DL- $\gamma$ -ornithine (48 mg, 0.24 mmole). Yield 55%; mp. 230-231°C.



5     *Scheme 4: Synthesis of tetraazatriphenylenes from 4,7-phenanthroline-4,5-quinone*

Example 5

Synthesis of hexaazatriphenylene analogues

10

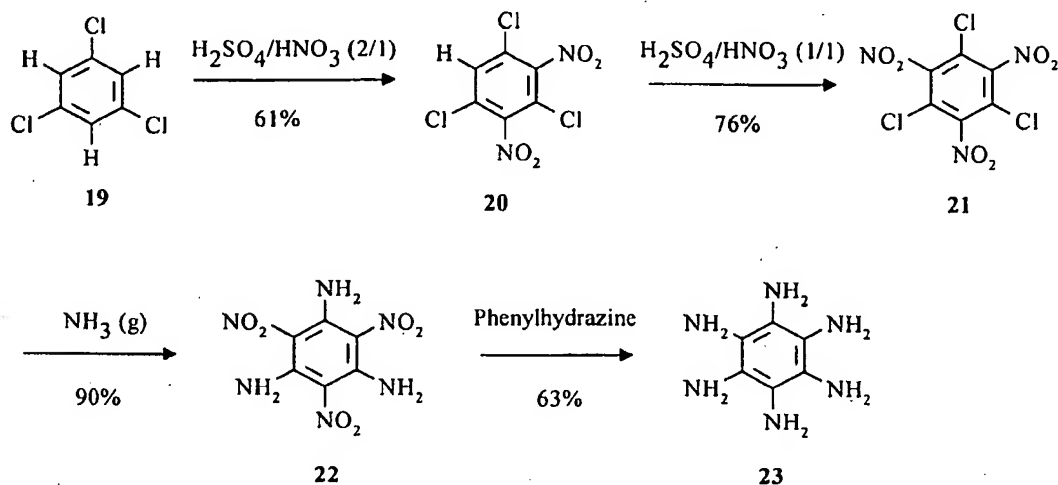
Hexamethyl-hexaazatriphenylene (26) and hexaphenyl-hexaazatriphenylene (27) were synthesized according to literature procedures (Kohne, B; Praefcke, K. *Liebigs Ann. Chem.*, 108 (1975), 875). The key step is the condensation of benzil (25) or 2,3-butanedione (24) with hexaaminobenzene (23), which was obtained by reduction of 1,3,5-triamino-2,4,6-trinitrobenzene (22) with phenylhydrazine. 1,3,5-Triamino-2,4,6-trinitrobenzene (22) was synthesized by nitration of 1,3,5-trichlorobenzene 19 to give 1,3,5-trichloro-2,4-dinitrobenzene (20). Harsh nitration conditions on dinitro derivative (20) with sulfuric acid/nitric acid gave 1,3,5-trichloro-2,4,6-trinitrobenzene (21) in

15



15

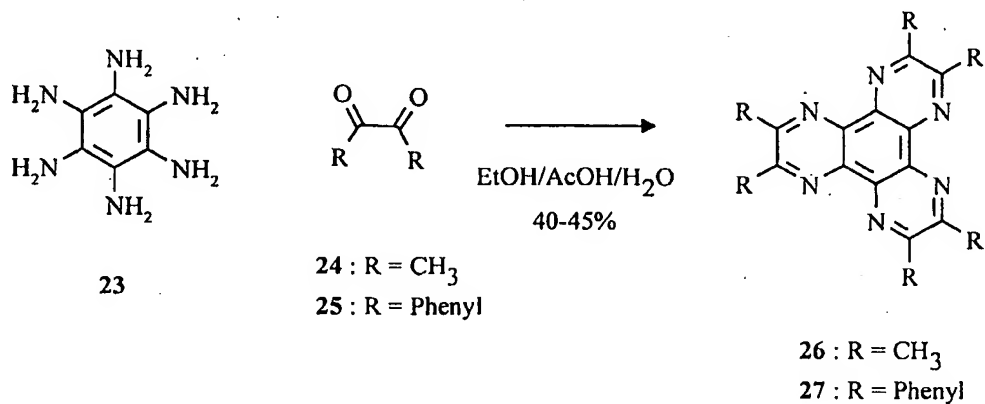
76% yield. Subsequent addition of  $\text{NH}_3$  gas to (21) gave strongly yellow triamine (22) in 90% yield. Reduction of the trinitro functionalities of 22 with phenylhydrazine gave hexaaminobenzene (23) in 63% yield.



5

*Scheme 5: Synthesis of Hexaaminobenzene 23*

Hexaaminobenzene 23 reacts with the  $\alpha$ -diketones benzil (25) and 2,3-butanedione (24) to give the 2,3,6,7,10,11-hexasubstituted dipyrazino[2,3-*f*:2',3'-*h*]quinoxalines (26) and (27) in 40-45% yield as depicted in Scheme 6.



*Scheme 6: Synthesis of Hexaazatriphenylene Derivatives 26, 27*

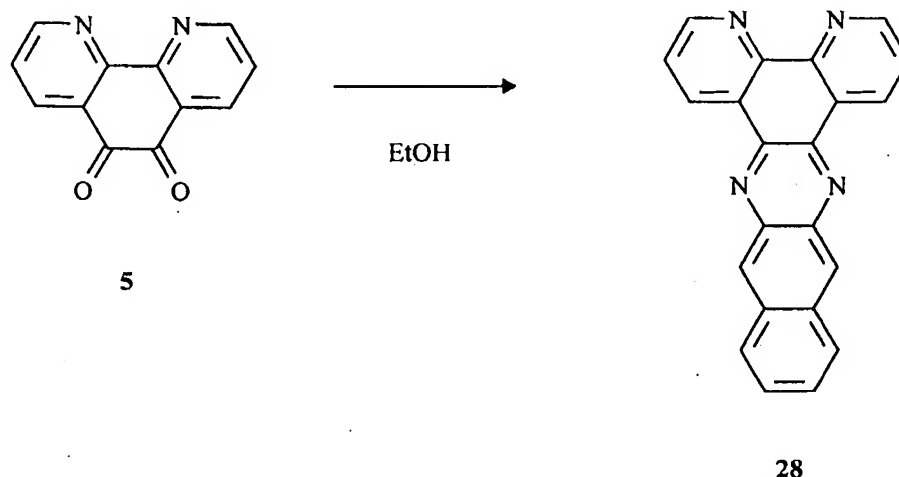
15

Example 6

Synthesis of further red-shifted tetraazatriphenylene from 1,10-phenanthroline-4,5-quinone

5 2,3-(2,3-diaminonaphthalene)-dipyrido[3,2-f:2',3'-h]quinoxaline(28).

Prepared similar to compound (6), starting from 1,10-phenanthroline-4,5-quinone (5) (0.6 g, 2.9 mmols) and 2,3-diaminonaphthalene(0.45 g, 2.9 mmols).



10 *Scheme 7: Synthesis of Tetraazatriphenylene Derivative 28*

Example 7

Synthesis of TBDMS-derivative (32)

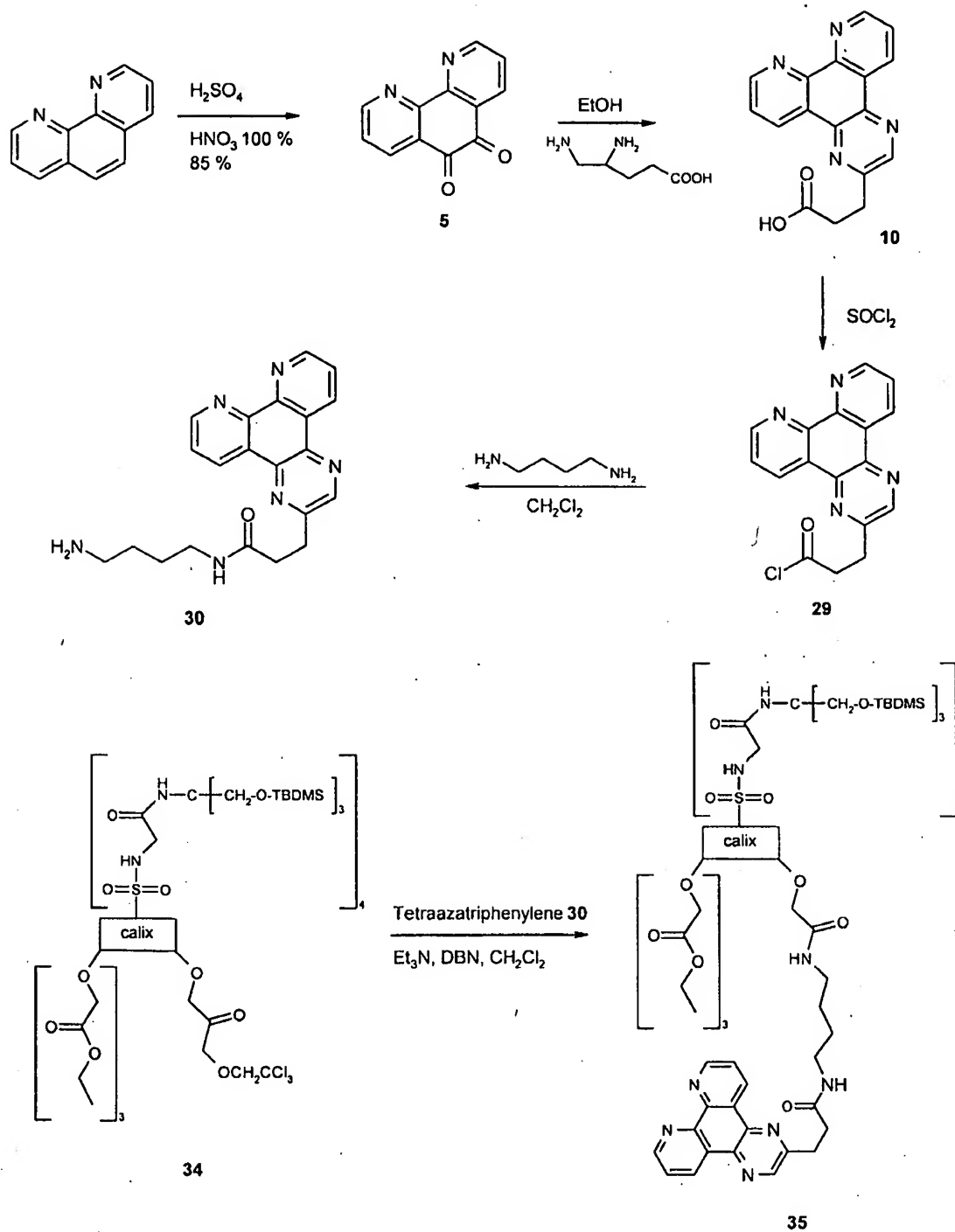
15

A solution of TBDMS-monotrichloroethanolcalix[4]arene (31) (260 mg, 0.081 mmole; prepared according to Scheme 8 as described in F.J. Steemers, Sensitizer-Modified Lanthanide Complexes for Time-Resolved Fluoroimmunoassays, Dissertation, University of Twente, The Netherlands  
 20 (1997), pp. 103-107), tetraazatriphenylene amine (30) (35 mg, 0.093 mmole), 125  $\mu$ l of 1,5 diazabicyclo[4.3.0]non-5-ene (DBN), and 10  $\mu$ l of triethylamine in 10 ml of dichloromethane was stirred overnight at room

temperature. The solvent was removed *in vacuo* and the reaction mixture was purified by preparative silica plate with 10% methanol/dichloromethane as eluent. Yield of calix[4]arene (32) was 14%.

5 A solution of potassium carbonate (16 mg, 0.12 mmole) in 3 ml of water was added to a refluxing solution of calix[4]arene (32) (40 mg, 0.012 mmole) in methanol (10 ml). After 1 h of refluxing, the mixture was poured into water (50 ml), after which the pH was adjusted to about 2 with 1 N hydrochloric acid. The water layer was extracted with dichloromethane to give, after removal of the solvent *in vacuo*, the triacid as a white solid. Yield  
10 50%. A solution of 0.5 g of this triacid calix[4]arene in trifluoroacetic acid (TFA/H<sub>2</sub>O; 9/1, 25 ml) was stirred overnight after which toluene (50 ml) was added. The solution was concentrated *in vacuo*, after which fresh toluene (50 ml) was added and the mixture was concentrated *in vacuo*. This procedure was repeated three times. The crude white solid was dissolved  
15 in a minimum amount of water and acetone was added dropwise to this solution till the product started to precipitate. After the suspension was stirred for 30 min, the product was filtered, freeze-dried, and analyzed. Prior to the luminescence measurements, the solution of this compound in water was dialyzed to remove excess of trifluoroacetic acid and was freeze-  
20 dried again.

18



Scheme 8

### Example 8

#### Preparation of Rare-Earth Complexes

Rare-earth complexes have been prepared for all compounds described above. The complexes have been obtained by adding a 1:2 (mole lanthanide/mole ligand) amount of lanthanide salt (generally the nitrate was used) to a solution of ligand in acetonitrile, which had been kept over molecular sieves prior to use. Titration experiments demonstrated that a 2:1 complex is formed between the ligand and the lanthanide ion. The luminescence of the sensitized lanthanide is at a maximum for the 2:1 composition of the complex. The following complexes have been prepared: Tb<sup>3+</sup>, Eu<sup>3+</sup>, Dy<sup>3+</sup>, Sm<sup>3+</sup>, Yb<sup>3+</sup>, Nd<sup>3+</sup>, and Er<sup>3+</sup>.

### Example 9

#### Photophysical measurements

For the complexes prepared as described in Example 8 absorption spectra and luminescence emission and excitation spectra have been recorded. In addition luminescence lifetime measurements have been done, in aerated and deaerated solution. Quantum yields were determined for the Tb- and Eu-complexes, using quinine sulfate in 1 M sulfuric acid (with a reported quantum yield of 0.546) as reference.

The effective sensitization of the lanthanide ions is clear from the luminescence excitation spectra: when the rare earth luminescence is monitored, variation of the excitation wavelength shows a spectrum which is similar to the absorption spectrum of the organic ligand. In most cases the absorption spectrum of the ligand is even enhanced and somewhat red-shifted upon complexation with the rare earth ion, which is favorable for most applications. Sensitized excitation results in a tremendous

improvement of the luminescence intensity, due to the typically 1,000-10,000 fold higher absorption cross sections of the ligand, as compared to the lanthanide ion. The energy transfer rate is high, since deaeration does not have any great impact on most measured luminescence intensities and lifetimes.

Quantitative data for a number of complexes are summarized in Tables 1-3.

Table 1. Photophysical data of (aza)triphenylenes and their  $\text{Eu}^{3+}$  and  $\text{Tb}^{3+}$  complexes in acetonitrile.

compound	$^1\text{E}_{00}$ ( $\text{cm}^{-1}$ ) (nm)	$\epsilon$ ( $\text{M}^{-1}\cdot\text{cm}^{-1}$ )	$^3\text{E}_{00}$ ( $\text{cm}^{-1}$ )	$\Phi_{\text{Eu}}$ ( $\lambda_{\text{ex}}$ )	$\Phi_{\text{Tb}}$ ( $\lambda_{\text{ex}}$ )
triphenylene	29900 (334)	355	23300	< 0.001	< 0.001
3	29700 (337)	1290	23800	0.41 (337)	0.55 (337)
8	28800 (347)	6440	23400	0.37 (343)	0.62 (343)
9	26500 (377)	9160	~18500	0	0
6	29400 (340)	3800	23800	0.41 (335)	0.67 (335)
7	27300 (366)	5520	21700	0.25 (355)	0.14 (355)
26	29800 (336) <sup>b</sup>	9300	22300	0.001 (345)	<0.001 (345)
27	27200 (367) <sup>b,c</sup>	>9000 <sup>c</sup>	~19100	0	0
16	28600 (350)	6630	22500	0.29 (340)	0.30 (340)
11	29400 (340)	8500	23600	0.43 (337)	0.51 (337)

<sup>a</sup> Not determined. <sup>b</sup> Shoulder on broad absorption. <sup>c</sup> Saturated solution.

<sup>d</sup> Abbreviations:

- $^1\text{E}_{00}$  = energy of the lowest electronic singlet 0-0 transition (determined from the absorption spectrum);
- $\epsilon$  = extinction coefficient of the electronic singlet 0-0 transition;
- $^3\text{E}_{00}$  = energy of the triplet 0-0 transition (determined from the 77 K phosphorescence spectrum);
- $\Phi$  = luminescence quantum yield, determined with reference to the quinine sulfate standard.

Table 2. Luminescence lifetimes of azatriphenylene-lanthanide complexes in acetonitrile;  $\lambda_{ex} = 308$  nm.

compound	$\tau_{Eu,ox}$ (ms)	$\tau_{Eu,deox}$ (ms)	$\tau_{Tb,ox}$ (ms)	$\tau_{Tb,deox}$ (ms)
3	1.20	1.19	1.31	1.32
8	1.03	1.02	1.20	1.21
6	1.04	0.98	1.43	1.36

<sup>a</sup> Abbreviations:  $\tau_{ox}$  is luminescence lifetime in oxygen containing solution,  $\tau^{deox}$  is luminescence lifetime in deoxygenated solution.

5

Compounds 9, 26, 27 and 28 have insufficient triplet energy to sensitize the  $Eu^{3+}$ - and  $Tb^{3+}$ -ions, which emit in the visible part of the spectrum and therefore require sensitization in the UV- or blue part of the spectrum. The "red-shifted" sensitizers, however, do give energy transfer to near-IR emitting rare earth ions like  $Yb^{3+}$ ,  $Nd^{3+}$ , and  $Er^{3+}$ .

10

Table 3. Photophysical data of (aza)triphenylene based  $Yb^{3+}$ ,  $Nd^{3+}$ , and  $Er^{3+}$  complexes in acetonitrile.

compound	${}^1E_{00}$ (cm <sup>-1</sup> ) (nm)	$\tau_{Yb,ox}$ ( $\mu$ s)	$\tau_{Nd,ox}$ ( $\mu$ s)	$\tau_{Er,ox}$ ( $\mu$ s)
3	29700 (337)	7.4	0.38	1.7
9	26500 (377)	6.2	0.33	2.0
6	29400 (340)	7.5	0.29	2.3
28	21500 (465)	7.9	0.32	2.5

<sup>a</sup> Abbreviations:

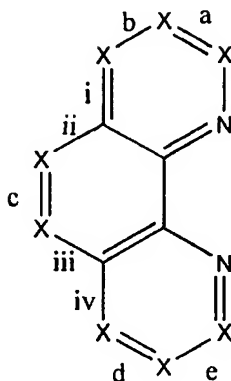
15

${}^1E_{00}$  = energy of the lowest electronic singlet 0-0 transition (determined from the absorption spectrum);

$\tau^{ox}$  ( $\mu$ s) = lifetime of the rare earth ion luminescence (longest, generally main, component) in oxygen-containing solution.

Claims:

1. A complex comprising a rare-earth metal ion and the complexing moiety of the general formula:



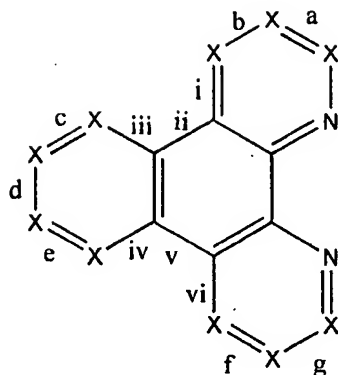
characterized in that

X is independently CH or N, and the bonds a, b, c, d, and e, and the combination of bonds i/ii and iii/iv are optionally condensed with a benzene group or a condensed aromatic moiety, wherein aromatic carbon atoms may be replaced by nitrogen atoms and wherein the complexing moiety may be substituted with C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>3-4</sub> alkylene, CN, halogen, COOH, C<sub>1-3</sub> alkyl-COOH, NO<sub>2</sub>, NH<sub>2</sub>, or a pending group for further functionalization or complexation, with the proviso that the complexing moiety is not 1,10-phenanthroline.

2. The complex of claim 1 wherein at least one the groups X is N.
3. The complex of claim 2, wherein two of the groups X are N.
4. The complex of claim 2 comprising a rare-earth metal ion and the complexing moiety of the general formula:



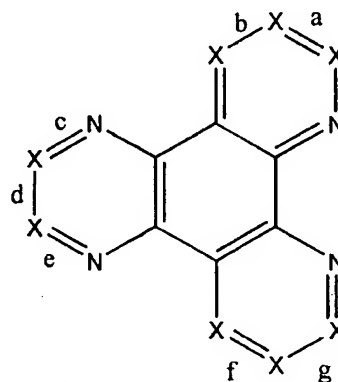
23



wherein X is independently CH or N, preferably at least one of the groups X being N, and the bonds a, b, c, d, e, f, and g, and the combination of bonds i/ii/iii and iv/v/vi are optionally condensed with a benzene group or a condensed aromatic moiety, wherein aromatic carbon atoms may be replaced by nitrogen atoms and wherein the complexing moiety may be substituted with C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>3-4</sub> alkylene, CN, halogen, COOH, C<sub>1-3</sub> alkyl-COOH, NO<sub>2</sub>, NH<sub>2</sub>, or a pending group for further functionalization or complexation.

10

5. The complex of claim 4 comprising a rare-earth metal ion and the complexing moiety of the general formula:



wherein X is independently CH or N, and the bonds a, b, c, d, e, f, and g are optionally condensed with a benzene group or a condensed aromatic moiety, wherein aromatic carbon atoms may be replaced by nitrogen atoms and wherein the complexing moiety may be substituted with C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>3-4</sub> alkylene, CN, halogen,

15

COOH, C<sub>1-3</sub> alkyl-COOH, NO<sub>2</sub>, NH<sub>2</sub>, or a pending group for further functionalization or complexation.

6. A process for preparing the complex of any one of claims 1-5 comprising  
5 the step of bringing into contact a solution of a rare-earth salt to a solution of the complexing moiety of claim 1.

7. An optical device or a diagnostic kit comprising the complex of any one of claims 1-5.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/02359

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07D471/04 C07D471/14 C09K11/00 C07D487/14  
 //(C07D471/04,221:00,221:00),(C07D471/14,241:00,221:00,221:00),  
 (C07D487/14,241:00,241:00,241:00)

According to International Patent Classification(IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D C09K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 109, no. 10, 5 September 1988 Columbus, Ohio, US; abstract no. 84977d, R.N. SHCHELOKOV ET AL.: "Rare earth nitrate complexes with 1,10-phenanthroline in the outer sphere" page 812; XP002041926 see abstract & ZH. NEORG. KHIM., vol. 33, no. 4, 1988, pages 867-74, --- -/--	1

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

31 August 1998

Date of mailing of the international search report

07/09/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
 Fax: (+31-70) 340-3016

Authorized officer

Alfaro Faus, I

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/02359

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 111, no. 18, 1989 Columbus, Ohio, US; abstract no. 163397j, E.F. GUDGIN TEMPELTON: "Spectroscopic characterization of 1,10-phenanthroline-2,9-dicarboxylic acid and its complexes with europium(III): luminescent europium chelates useful for analytical applications" page 656; XP002041927 see abstract & J. LUMIN., vol. 43, no. 4, 1989, pages 195-205, -----	1,7
A	EP 0 171 978 A (HSC) 19 February 1986 see claim 7 -----	1,7

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 98/02359

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 171978 A	19-02-1986	CA 1254829 A	30-05-1989
		FI 853090 A	14-02-1986
		US 4772563 A	20-09-1988
		JP 61200988 A	05-09-1986
<hr/>			